Supporting Information

The iron-sulfur cluster of pyruvate formate-lyase activating enzyme in whole cells: cluster interconversion and a valence-localized $[4Fe\text{-}4S]^{2+}$ state

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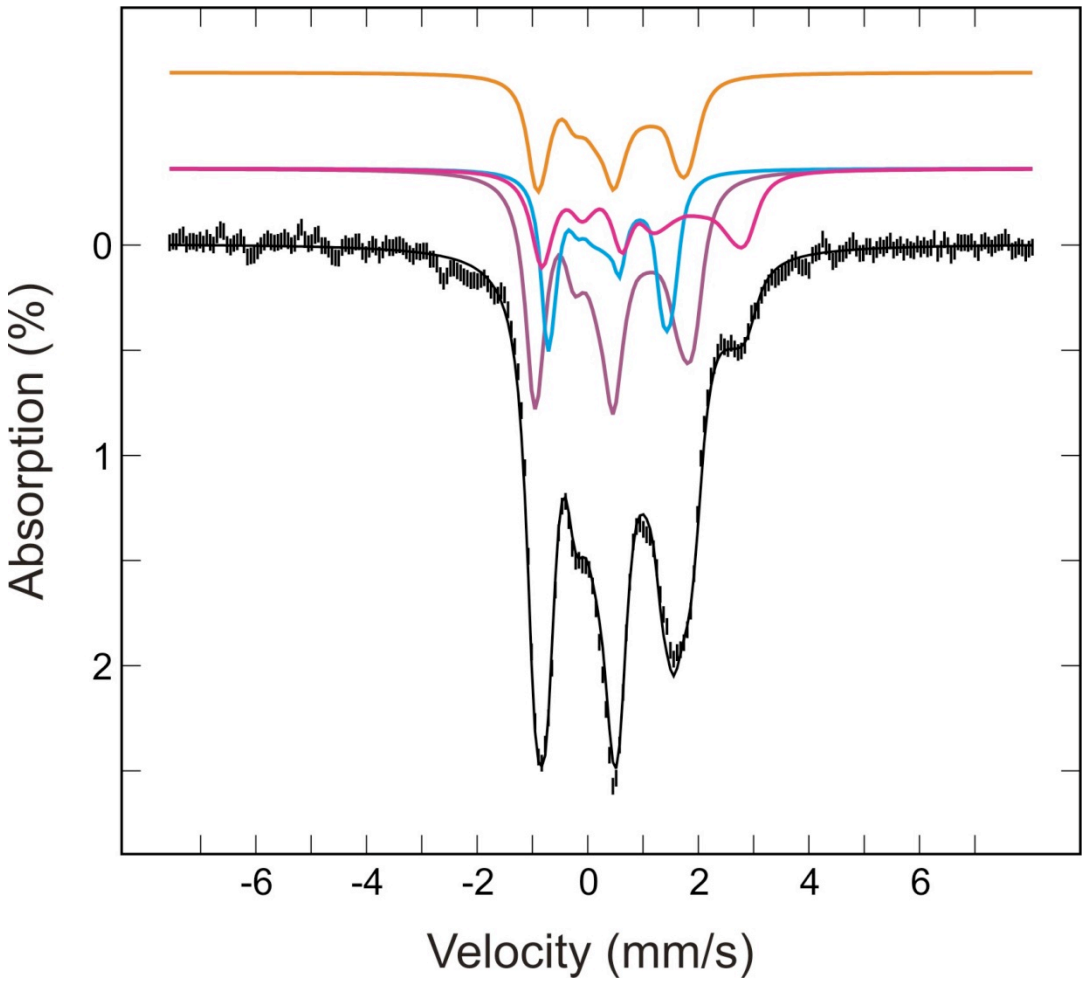

Figure S1. High-field Mössbauer spectrum of purified PFL-AE (0.64 mM) in the presence of 5'-deoxyadenosine (6.4 mM). The spectrum (hatched marks) was recorded at 4.2 K in a parallel field of 6 T. Contributions from the Fe^{II} impurity (~4%) has been removed from the raw data. The solid black line overlaid with the experimental spectrum is the sum of the theoretical component spectra simulated by using the parameters given in the text. The simulated component spectra are color coded for the valance delocalized $Fe^{II}Fe^{III}$ pair (purple), the Fe^{III} site (cyan) and the LOC Fe^{II} site (magenta) of the bound $[4Fe\text{-}4S]^{2+}$ cluster and the "unboud" $[4Fe\text{-}4S]^{2+}$ cluster (orange). Percent absorption for the bound and unbound clusters are 77% and 19%, respectively.

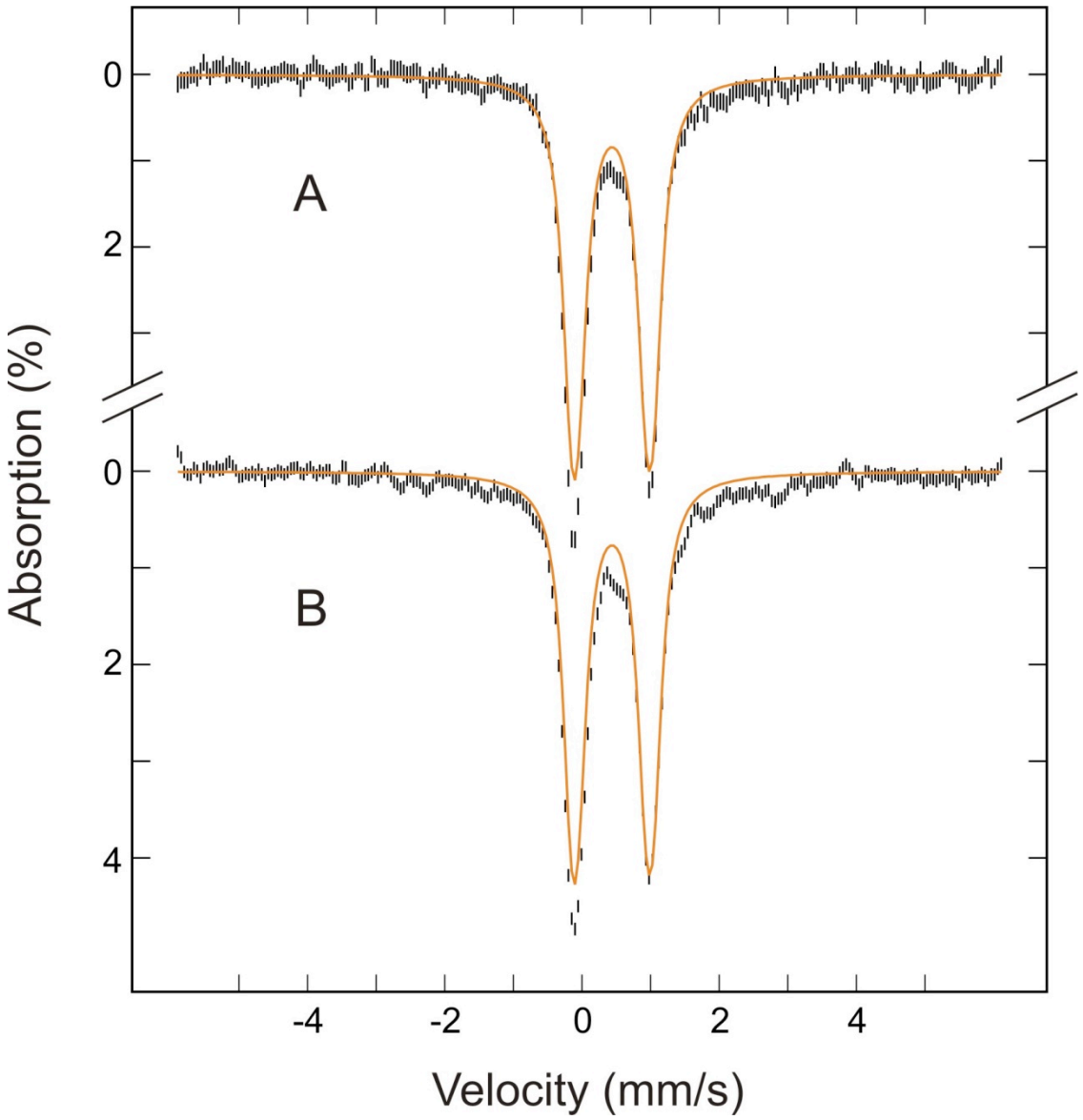

Figure S2. Representative Mössbauer spectra of purified PFL-AE (0.64 mM) in the presence of small molecules (6.4 mM) that do not induce valence localization: (A) PFL-AE plus pyruvate, and (B) PFL-AE plus ATP. The spectra (hatched marks) were recorded at 4.2 K in a parallel magnetic field of 50 mT. For comparison, the simulated spectrum of the as-purified PFL-AE in the absence of small molecules (orange lines) is overlaid with the experimental spectra.

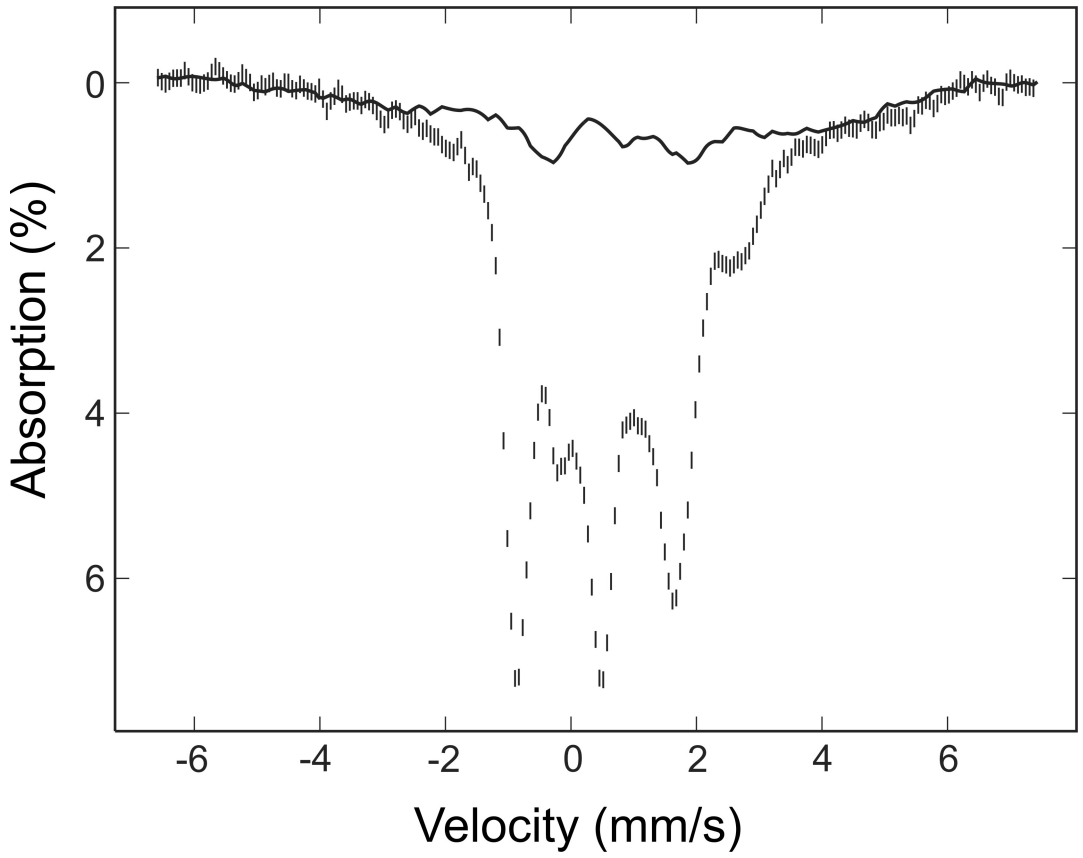

Figure S3. Mössbauer spectrum of the 16-hour anaerobic growth cells recorded at 4.2 K in a parallel applied field of 6T. In an attempt to remove the contributions from the non-cluster Fe^{II}, we search our data bank for a spectrum of an adventitiously bound Fe^{II} species that exhibits broad absorptions resembling that of the non-cluster Fe^{II}. The solid line is the spectrum of adventitiously bound Fe^{II} in a sample containing anaerobically purified recombinant *Azotobacter vinlandii* Fe protein of the nitrogenase system. The adventitiously-bound-Fe^{II} spectrum is normalized to 26% of the total absorption of the whole cell spectrum. Removal of this contribution yields the spectrum shown in Figure 3B.